

**WHAT WE CLAIM IS:**

1. A method for inducing gene silencing effects using sense DNA and antisense RNA (cDNA-aRNA) hybrids, comprising the steps of:
  - a. providing a plurality of DNA sequences, wherein said DNA sequences are homologous to a or a plurality of targeted intracellular messenger RNA sequences;
  - b. contacting said DNA sequences to a plurality of RNA sequences to form a plurality of DNA-RNA hybrids, wherein said RNA sequences are complementary to said DNA and intracellular messenger RNA sequences; and
  - c. transducing said DNA-RNA hybrids into a plurality of cells which are sensitive to RNA interference effects; and so as to provide a specific gene silencing effect to the targeted messenger RNAs within said cells.
2. The method as defined in Claim 1, further comprising the generation of said DNA sequences by a machine selected from the group consisting of oligonucleotide synthesizer, thermocycler and isothermal incubator.
3. The method as defined in Claim 1, further comprising the generation of said DNA sequences from a plurality of template vectors by enzymatic methods selected from the group consisting of reverse transcription, polymerase chain reaction, nucleic acid sequence based amplification, and RNA-polymerase cycling reaction.
4. The method as defined in Claim 3, wherein said template vectors are nucleic acid sequences containing said DNA sequences in the form of single-stranded, double-stranded, linear and/or circular structures.

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5. The method as defined in Claim 1, wherein said DNA sequences are completely homologous to said intracellular messenger RNA sequences.
  6. The method as defined in Claim 1, wherein said DNA sequences are partially homologous to said intracellular messenger RNA sequences.
  7. The method as defined in Claim 6, wherein said DNA sequences contain a plurality of nucleotide analogs selected from the group consisting of inosine, xanthine, hypoxanthine and their derivative analogs.
  8. The method as defined in Claim 1, further comprising the generation of said RNA sequences by a machine selected from the group consisting of oligonucleotide synthesizer, thermocycler and isothermal incubator.
  9. The method as defined in Claim 1, further comprising the generation of said RNA sequences from a plurality of template vectors by enzymatic methods selected from the group consisting of in-vitro transcription, aRNA amplification, nucleic acid sequence based amplification, and RNA-polymerase cycling reaction.
  10. The method as defined in Claim 9, wherein said template vectors are nucleic acid sequences containing said RNA sequences in the form of single-stranded, double-stranded, linear and/or circular structures.
  11. The method as defined in Claim 1, wherein said RNA sequences are completely complementary to said DNA sequences.
  12. The method as defined in Claim 1, wherein said RNA sequences are partially complementary to said DNA sequences.
  13. The method as defined in Claim 1, further comprising the hybridization of said DNA and RNA sequences to form duplex sequences.

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14. The method as defined in Claim 13, wherein said DNA and RNA sequences are hybridized in a Hepes-containing buffer at about 68°C for more than 10 minutes.
  15. The method as defined in Claim 14, wherein said Hepes-containing buffer is 20 mM HEPES solution.
  16. The method as defined in Claim 13, wherein said duplex sequences are completely matched.
  17. The method as defined in Claim 13, wherein said duplex sequences are partially matched.
  18. The method as defined in Claim 17, wherein said duplex sequences contain mismatched base pairs selected from the group consisting of inosine, xanthine, hypoxanthine and their derivative analogs.
  19. The method as defined in Claim 1, further comprising the transduction of said DNA-RNA hybrids into said cells by a method selected from the group consisting of micro-injection, liposomal transfection, calcium phosphate transfection, chemical transformation, and electroporation.
  20. The method as defined in Claim 1, wherein said cells contain RNA-directed endoribonucleases (RDE) for inducing RNA interference effects by the derivatives of said RNA sequences in said cells.
  21. The method as defined in Claim 20, wherein said RNA-directed endoribonuclease are naturally possessed by the said cells or introduced recombinantly into the said cells.
  22. A method for generating cDNA-aRNA hybrids for gene silencing, comprising the steps of:

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- a. providing: i) a solution comprising a nucleic acid template, ii) one or more primers sufficiently complementary to the sense conformation of the nucleic acid template, and iii) one or more promoter-linked primers sufficiently complementary to the antisense conformation of the nucleic acid template, and having an RNA promoter;
  - b. treating the nucleic acid template with one or more primers under conditions such that a first cDNA strand is synthesized;
  - c. treating the first cDNA strand with one or more promoter-linked primers under conditions such that a plurality of promoter-linked double-stranded nucleic acid templates are synthesized;
  - d. treating the promoter-linked double-stranded nucleic acid templates under conditions such that essentially aRNA fragments are synthesized; and
  - e. treating aRNA fragments with one or more primers under conditions such that a plurality of cDNA-aRNA hybrids are synthesized.
23. The methods as defined in Claim 22, can comprise the step of repeating steps b) through e) for a sufficient number of cycles to obtain a desired amount of amplified hybrid product.
24. The methods as defined in Claim 22, the treating step in step b) can comprise heating the solution at a temperature above 90°C to provide denatured nucleic acids.
25. The methods as defined in Claim 22, the treating step in step c) can comprise treating the first cDNA strand with one or more promoter-linked primers at a temperature ranging from about 37°C to about 72°C.

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26. The methods as defined in Claim 22, the treating step in step c) can also comprise treating the cDNA strand with one or more promoter-linked primers in the presence of a plurality of polymerases.
  27. The methods as defined in Claim 26, wherein said polymerase is selected from the group consisting of DNA-dependent DNA polymerases, RNA-dependent DNA polymerases, RNA polymerases, Taq-like DNA polymerase, Tth-like DNA polymerase, *C. therm.* polymerase, viral replicases, and combinations thereof. The viral replicases can be selected from the group consisting of avian myeloblastosis (AMV) reverse transcriptase and Moloney murine leukemia virus (MMLV) reverse transcriptase. In particular, the AMV reverse transcriptase does not have RNase H activity.
  28. The methods as defined in Claim 22, the treating step in step d) further comprise treating the promoter-linked double-stranded nucleic acid with an enzyme having transcriptase activity at about 37°C.
  29. The methods as defined in Claim 28, wherein said enzyme having transcriptase activity can be selected from the group consisting of RNA polymerases and viral replicases.
  30. The methods as defined in Claim 29, wherein said RNA polymerases can be selected from the group consisting of T3 RNA polymerase, T7 RNA polymerase, SP6 RNA polymerase, and M13 RNA polymerase.
  31. The methods as defined in Claim 29, wherein said viral replicases can be selected from the group consisting of avian myeloblastosis (AMV) reverse transcriptase and Moloney murine leukemia virus (MMLV) reverse transcriptase. In particular, the AMV reverse transcriptase does not have RNase H activity.

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32. The methods as defined in Claim 22, wherein said primers are complementary to the 3'-ends of the antisense conformation of the nucleic acid template, comprising a sequence-specific primer homologous to the targeted gene transcript.
  33. The methods as defined in Claim 22, wherein said promoter-linked primers are complementary to the 3'-ends of the sense conformation of the nucleic acid template, comprising a sequence-specific primer complementary to the targeted gene transcript, such as T7 promoter-linked poly(dT) primers.
  34. The methods as defined in Claim 22, wherein said promoter-linked double-stranded nucleic acid template can be selected from the group consisting of linear and circular promoter-containing double-stranded DNAs or promoter-linked partial single-stranded DNAs.
  35. The methods as defined in Claim 22, the treating step in step e) comprises treating aRNA fragments with one or more primers at a temperature ranging from about 37°C to about 70°C.
  36. The methods as defined in Claim 22, further comprising the step of incorporating one or more nucleotide analogs into the cDNA portion of the cDNA-aRNA hybrid to facilitate the onset of RNAi-related effects.
  37. The methods as defined in Claim 36, wherein said nucleotide analog is selected from the group consisting of inosine, xanthine, hypoxanthine and their derivative nucleotides.
  38. The methods as defined in Claim 22, further comprising the step of contacting cDNA-aRNA hybrids with a reagent for gene silencing transfections.

39. The methods as defined in Claim 38, wherein said reagent can be selected from the group consisting of electroporesis media, chemical transduction reagents and liposomal transfection reagents.
40. A method of improved RNA-polymerase cycling reaction which amplifies a specific DNA-RNA hybrid construct for transducing biological gene silencing effects, comprising the steps of:
- a. providing a plurality of nucleic acid sequences as an amplifiable gene template for following reactions;
  - b. denaturing and contacting said nucleic acid template with a plurality of primers and a plurality of promoter-linked primers, wherein said primers and promoter-linked primers are respectively complementary to the sense and antisense sequence conformation of said nucleic acid template;
  - c. permitting extension of said primers and promoter-linked primers to form a plurality of promoter-linked double-stranded nucleic acid sequences, wherein said promoter-linked double-stranded nucleic acid sequences are formed by either DNA-directed or RNA-directed DNA and/or RNA polymerases or the combination thereof;
  - d. permitting transcription of said promoter-linked double-stranded nucleic acid sequences to form a plurality of amplified RNA fragments, wherein said amplified RNA fragments are generated by extension of RNA polymerase activity through the promoter region of said promoter-linked double-stranded DNAs; and
  - e. contacting said amplified RNA fragments with said primer to form a plurality of DNA-RNA hybrid duplexes, wherein said DNA-RNA hybrid

duplexes are formed by reverse transcription of said amplified RNA fragments with the extension of said primer; so as to provide amplified cDNA-aRNA hybrids for gene silencing effects.

41. The method as defined in Claim 40, further comprising repeated steps (b) through (e) on said amplified DNA-RNA hybrids at least one time.
42. The method as defined in Claim 40, further comprising the step of generating and/or incorporating a plurality of nucleotide analogs into the cDNA part of said amplified cDNA-aRNA hybrids in the step (e) for the increase of onset of gene silencing effects.
43. The method as defined in Claim 42. wherein said nucleotide analog is generated by deaminase.
44. The method as defined in Claim 42. wherein said nucleotide analog is generated by chemical treatments.
45. The method as defined in Claim 42. wherein said nucleotide analog is selected from the group consisting of inosine, xanthine, hypoxanthine and their derivative analogs.
46. The method as defined in Claim 40, further comprising the step of mixing said amplified cDNA-aRNA hybrids with a plurality of reagents after step (e) for gene silencing or knockout transfection.
47. The methods as defined in Claim 46, wherein said reagent can be selected from the group consisting of electroporesis media, chemical transduction reagents and liposomal transfection reagents.

48. The method as defined in Claim 40, wherein said nucleic acid template need to be denatured at temperature ranged from about 90°C to about 100°C in the step (b).
  49. The method as defined in Claim 40, wherein said promoter-linked double-stranded nucleic acid sequences are selected from the group consisting of linear and circular forms of promoter-linked double-stranded DNAs and promoter-linked single-stranded DNAs.
  50. The method as defined in Claim 40, wherein said DNA-directed or RNA-directed DNA and/or RNA polymerases are enzyme activities selected from the group consisting of AMV reverse transcriptase without RNase H activity/Taq-like DNA polymerase mixtures, thermostable MMLV reverse transcriptase/ Taq-like DNA polymerase mixtures, Tth-like DNA polymerases with reverse transcription activity, *C. therm.* polymerases, and RNA polymerase/replicase mixture.
  51. The method as defined in Claim 50, wherein said enzyme activities are performed at temperature ranged from about 37°C to about 70°C.
  52. The method as defined in Claim 40, wherein said primers are complementary to the 3'-ends of the antisense conformation part of said nucleic acid template.
  53. The method as defined in Claim 40, wherein said promoter-linked primers are complementary to the 3'-ends of the sense conformation part of said nucleic acid template.
  54. The method as defined in Claim 40, wherein said RNA polymerase or replicase activity is an enzyme activity selected from the group consisting of T3, T7, SP6 and M13 RNA polymerase and viral replicases.
  55. The method as defined in Claim 54, wherein said RNA polymerase activity is performed at temperature ranged about 37°C.

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56. The method as defined in Claim 40, wherein said cDNA-aRNA hybrid duplexes are formed by the group consisting of AMV reverse transcriptases without RNase H activity, thermostable MMLV reverse transcriptases, Tth-like DNA polymerases with reverse transcription activity and *C. therm.* polymerases.
  57. The method as defined in Claim 56, wherein said cDNA-aRNA hybrid duplexes are formed at temperature ranged from about 37°C to about 70°C.
  58. The method as defined in Claim 40, wherein said cDNA-aRNA hybrid duplexes are selected from the group consisting of pathogenic nucleic acids, viral genes, mutated genes, oncogenes, known functional genes or unknown functional nucleic acid sequences.
  59. A kit for inducing gene silencing effects using cDNA-aRNA hybrids, comprising the components of:
    - a. a plurality of cDNA-aRNA hybrid constructs, wherein the cDNA portion of said cDNA-aRNA hybrid constructs are homologous to a or a plurality of targeted intracellular messenger RNA sequences; and
    - b. a plurality of transfection reagents, wherein said transfection reagents can deliver said cDNA-aRNA hybrid constructs into a plurality of targeted cells; and so as to provide a specific gene silencing effect to the targeted messenger RNAs within said cells.
  60. The kit as defined in Claim 59, further comprising the generation of said cDNA-aRNA hybrid constructs by a machine selected from the group consisting of oilgonucleotide synthesizer, thermocycler and isothermal incubator.
  61. The kit as defined in Claim 59, further comprising the generation of said cDNA-aRNA hybrid constructs from a plurality of template vectors by enzymatic

- methods selected from the group consisting of reverse transcription, polymerase chain reaction, nucleic acid sequence based amplification, and RNA-polymerase cycling reaction.
62. The kit as defined in Claim 61, wherein said cDNA-aRNA hybrid constructs are generated by RNA-polymerase cycling reaction (RNA-PCR).
  63. The kit as defined in Claim 61, wherein said template vectors are nucleic acid sequences containing the cDNA portion of said cDNA-aRNA hybrid constructs in the form of single-stranded, double-stranded, linear and/or circular structures.
  64. The kit as defined in Claim 59, wherein said cDNA-aRNA hybrid constructs are completely homologous to said intracellular messenger RNA sequences.
  65. The kit as defined in Claim 59, wherein said cDNA-aRNA hybrid constructs are partially homologous to said intracellular messenger RNA sequences.
  66. The kit as defined in Claim 59, wherein said cDNA-aRNA hybrid constructs contain a plurality of nucleotide analogs selected from the group consisting of inosine, xanthine, hypoxanthine and their derivatives.
  67. The kit as defined in Claim 59, further comprising the hybridization of a plurality of DNA and RNA sequences to form said cDNA-aRNA hybrid constructs.
  68. The kit as defined in Claim 67, wherein said DNA and RNA sequences are completely complementary to each other.
  69. The kit as defined in Claim 67, wherein said DNA and RNA sequences are partially complementary to each other.

70. The kit as defined in Claim 67, wherein said DNA and RNA sequences contain mis-matched base pairs selected from the group consisting of inosine, xanthine, hypoxanthine and their derivatives.
71. The kit as defined in Claim 59, wherein said transfection reagent can be selected from the group consisting of electroporesis media, chemical transduction reagents and liposomal transfection reagents.
72. The kit as defined in Claim 59, further comprising the transduction of said cDNA-aRNA hybrids into said targeted cells by a delivery method selected from the group consisting of micro-injection, liposomal transfection, calcium phosphate transfection, chemical transformation, vector penetration and electroporesis.
73. The kit as defined in Claim 59, wherein said targeted cells contain RNA-directed endoribonucleases for inducing RNA interference effects by the derivatives of said cDNA-aRNA hybrids in said cells.
74. A composition comprises a plurality of DNA-RNA hybrids, wherein said DNA-RNA hybrids are capable of inducing sequence-specific gene silencing or knockout effects in cells or organisms.
75. The composition as defined in Claim 74, wherein said cells are of high vertebrate origins.
76. The cells as defined in Claim 75, wherein said cells are selected from a group of cultured tissue cells comprising mouse, rat, rabbit, canine, chicken and human cells.
77. The composition as defined in Claim 74, wherein said organisms are mammalian origins.

78. The organisms as defined in Claim 77, wherein said organisms are selected from a group of animals comprising mouse, rat, rabbit, canine and human beings.
  79. A therapeutic strategy, measurement or treatment for human diseases using DNA-RNA hybrid construct as claimed in Claim 59 and/or 74.
  80. The therapeutic treatment as defined in Claim 79 is used for treating human tumors and cancers.
  81. The therapeutic treatment as defined in Claim 79 is used for treating viral infections.
  82. The virus infections as defined in Claim 81 are selected from a group of HIV, HCV, common cold Rhinovirus, Herpes virus, CMV, Ebola virus, oncogenic retrovirus, and other human-disease-inducing and/or -associated virus infections.
  83. A strategy for gene-based research and therapy using cDNA-RNA hybrid transfection against oncogene expression.
  84. A strategy for gene-based therapy using cDNA-RNA hybrid transfection against human immunodeficiency virus (HIV) gene expression and/or infection.
  85. A process uses a composition as defined in Claim 74.
  86. The said process in Claim 85 is useful for gene function analysis, drug discovery, drug target identification and drug target validation.
  87. A cell line derived from application of a composition as defined in Claim 74 or utilizes a process as defined in Claim 85.
  88. An organism derived from application of a composition as defined in Claim 74 or utilizes a process as defined in Claim 85.

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89. Information and informational data generated from a process as claimed in Claim 85, or derived from phenotypical and/or genotypical observations of a cell line of Claim 87 and/or an organism of Claim 88.
  90. A gene sequence useful for therapeutic intervention or treatment and diagnostics and prognostics as revealed with the application a composition as claimed in Claim 74 and/or the use of a process as claimed in Claim 85.
  91. A protein product derived from the said sequence in Claim 90 is useful for therapeutic intervention or treatment and diagnostics and prognostics.
  92. A drug target useful for screening therapeutic small molecule chemical drugs and antibody drugs and as diagnostic and prognostic marker as revealed with the application a composition as claimed in Claim 74 and/or the use of a process as claimed in Claim 85.
  93. A therapeutic drug for human and animal diseases derived from using a method of Claim 1, a composition of Claim 74, a process of Claim 85, a cell line of Claim 87, an organism of Claim 88, informational data of Claim 89, a gene sequence of Claim 90, a protein of Claim 91 or a drug target of Claim 92.
  94. A diagnostic or prognostic marker for human and animal diseases derived from using a method of Claim 1, a composition of Claim 74, a process of Claim 85, a cell line of Claim 87, an organism of Claim 88, informational data of Claim 89, a gene sequence of Claim 90, a protein of Claim 91 or a drug target of Claim 92.